

## **Aim - Determination of Total Available Carbohydrates from tissue (Clegg-Anthrone)**

**Principle:** This method determines the total amount of carbohydrates, based on hydrolyzable starch and soluble sugar content.

### **Reagents**

- 52% perchloric acid solution. 279 ml perchloric acid (specific gravity 1.70) in 100 ml distilled water. Let cool before using.
- Sulphuric acid solution. 760ml of H<sub>2</sub>SO<sub>4</sub> (specific gravity 1.84) in 330 ml distilled water. Let cool before using.
- Anthrone reagent. Have enough Anthrone reagent ready by preparing a 0.1% sulphuric acid solution to use the same day.
- Standard glucose solution. Dissolve 100 mg glucose in 100 ml water.
- Standard diluted glucose solution. Dilute 10 ml standard glucose solution in 100 ml distilled water (1 ml = 0.1 mg glucose).

### **Materials:**

- Spectrophotometer.
- Whatman N°542 or Schleicher and Schill N°150 filter paper.

### **Method:**

#### **Extraction:**

- Weigh out 1.0 g of dry or 2.5 g of wet sample to within 0.001 g containing approximately 60 to 300 mg of total available carbohydrates.
- Transfer quantitatively to a stoppered 100 ml graduated cylinder.
- Add 10 ml water and stir with glass rod to disperse the sample.
- Add 13 ml of the perchloric acid solution. Stir constantly with glass rod for 20 min.
- Rinse rod with distilled water and bring volume to 100 ml. Mix and filter into a 250 ml volumetric flask.
- Rinse the graduated cylinder with distilled water and add this to the volumetric flask. Calibrate the flask with distilled water and shake.

**Determination:**

- Dilute 10 ml of the extract to 100 ml with distilled water. Use a pipette to transfer 1 ml of diluted filtrate to a test-tube
- Using a pipette take two 1 ml samples of distilled water for duplicate blanks and put each one in a test-tube.
- Take two 1 ml duplicate blanks using the dilute glucose solution.
- Quickly add freshly prepared anthrone reagent to all the tubes. Cap the tubes and mix vigorously. Place in a water-bath and heat for 12 minutes.
- Cool rapidly to room temperature. Transfer the solution to 1 cm spectrophotometer cells. The green colour is stable for only 2 hours.
- Read the absorption rate at 630 nm against the blank.

**Calculation:****Where,**

W = Weight of sample

A = Absorption of diluted standard <sup>(1)</sup>

(1) The graph is a straight line in the range of 0.0–0.15 mg glucose (manual) and 0.0 – 1.5 mg glucose (automatic).